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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification 5 : A61M 31/00</p>	<p>A1</p>	<p>(11) International Publication Number: WO 93/19804 (43) International Publication Date: 14 October 1993 (14.10.93)</p>
<p>(21) International Application Number: PCT/US93/02228 (22) International Filing Date: 2 March 1993 (02.03.93) (30) Priority data: 07/844,497 2 March 1992 (02.03.92) US (71) Applicant: CRYOLIFE, INC. [US/US]; 2211 New Market Parkway, Marietta, GA 30067 (US). (72) Inventors: MORSE, Brenda, Smith ; 1703 Hart Run, Chamblee, GA 30341 (US). TURNER, A., Denise ; 1511 Summit Spring Drive, Dunwoody, GA 30350 (US). McNALLY, Robert, T. ; 4693 Karls Gate Drive, Marietta, GA 30068 (US).</p>		<p>(74) Agents: WILSON, Mary, J. et al.; Nixon & Vanderhye, 8th Floor, 1100 North Glebe Road, Arlington, VA 22201-4714 (US). (81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report</i> <i>With amended claims</i></p>
<p>(54) Title: FIBRIN SEALANT DELIVERY METHOD</p>		
<p>(57) Abstract</p> <p>The present invention relates to a method for the formulation of fibrin sealant in a single delivery system. The method involves mixing a fibrinogen/Factor XIII precipitate solution with thrombin under conditions such that thrombin clotting activity is inhibited and said mixture is applied to a body site under conditions which activate the thrombin to convert fibrinogen into fibrin sealant. A single device, syringe or container, can be used to apply the fibrin sealant formulation.</p>		

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FIBRIN SEALANT DELIVERY METHOD

BACKGROUND OF THE INVENTION

This is a continuation-in-part application
of Serial No. 07/460,379 filed on January 3, 1990,
5 which is hereby incorporated herein in its entirety.

Technical Field

The present invention relates to a method
of fibrin sealant preparation and delivery, which
permits use of a single delivery device. The method
10 may be used for autologous, single-donor, pooled-
donor or cell culture-derived fibrin sealant for
various human and veterinary surgical procedures.
The invention further relates to a kit suitable for
use in such a method.

15

BACKGROUND INFORMATION

The blood coagulation system is a complex
series of proteins and factors which are activated
sequentially to produce a fibrin gel or clot. In
the final stages of the process, fibrinogen is
20 cleaved by thrombin to generate fibrin monomer,
which rapidly polymerizes and is cross-linked by
activated Factor XIII to form a fibrin matrix.

Preparations of human coagulation factors,
including fibrinogen and thrombin, have been used
25 extensively in surgery over the last ten years
(Schlag et al (eds), Fibrin Sealant in Operative
Medicine, vol 1-7, Springer-Verlag, Heidelberg).
These biological fibrin sealants promote hemostasis
and wound healing by sealing leakage from tissues,
30 sutures, staples, and prostheses, and are

particularly useful during open heart surgery in
heparinized patients. The sealants also have use as
an adhesive for the bonding of tissues and they
reduce the amount of blood required for transfusions
by controlling intraoperative bleeding. Their
effectiveness is reflected in the extensive range of
surgical applications for which they have been used,
including cardiovascular surgery, plastic surgery,
orthopedics, urology, obstetrics and gynecology,
dentistry, maxillofacial and ophthalmic surgery.

Fibrin sealant products prepared from
pooled human plasma fibrinogen/Factor XIII are
available commercially in Europe (Tissucol/Tisseel,
Immuno AG, Vienna, Austria and Beriplast P, Hoechst,
West Germany) but such products have not received
U.S. Food and Drug Administration approval. As an
alternative, some hospitals are preparing fibrin
sealant in-house using the patient's own blood
(autologous) or single-donor (homologous) plasma as
a source of fibrinogen and Factor XIII.

The plasma fibrinogen/Factor XIII
component of fibrin sealant is typically prepared by
freezing plasma at a temperature below -20°C
overnight, slowly thawing the material at $0-4^{\circ}\text{C}$,
centrifuging, and transferring the cryoprecipitate
to a syringe or spray container (Dresdale et al,
Ann. Thorac. Surg. 40:385 1985; and U.S. Patent
4,627,879). The thrombin component, usually
purified from bovine plasma, can be obtained
commercially and is typically prepared in a separate
syringe or spray container. In use, the two
solutions are delivered simultaneously or
alternately to generate fibrin sealant at the site

of the wound; alternatively, the sealant is applied to a collagen matrix (e.g. Gelfoam or Avitene) and then pressed against the site (Lupinetti et al, J. Thorac. Cardiovasc. Surg. 90:502 1985; and U.S. Patent 4,453,939).

The use of fibrin sealant in surgery has been limited by problems associated with mixing and delivery of the sealant to the wound. Generation of fibrin sealant at the wound site is currently achieved using a two syringe or spray container system to prevent premature mixing and clotting of the components. Such two syringe systems are, however, unsatisfactory due to the awkwardness of filling and manipulating the delivery devices at the wound site. In addition, the syringe system is accompanied by problems of inadequate mixing of the two solutions, resulting in the formation of a weak clot. Alternatively, the two syringes can be placed into a holder designed such that the solutions are permitted to mix before entering the needle (U.S. Patents 4,735,616, 4,359,049, and 4,631,055). The mixing chamber directs the flow of the separate components through two narrow channels and forces mixing at the top of the outflow needle. Although the strength of the clot obtained using this method is reproducible, the needle frequently clogs and must repeatedly be replaced.

In view of the problems inherent in the methodologies currently available for delivering fibrin sealant, the need for a simple, reproducible technique is clear. Such a technique must be convenient to use and must result in the formation, at a specific site, of a clot of appropriate

strength. Such a delivery technique is provided by the invention disclosed herein.

SUMMARY OF THE INVENTION

5 It is a general object of the present invention to provide a method of forming a fibrin sealant from blood coagulation components that overcomes the problems associated with methods known in the art.

10 It is a specific object of the invention to provide a method of delivering fibrin sealant to a wound site, in which method a fibrinogen/Factor XIII-enriched precipitate (or a fibrinogen/Factor XIII mixture) and thrombin are mixed together under conditions such that clotting is prevented until
15 such time as sealant formation is desired.

It is another object of the present invention to provide means of reversibly inactivating thrombin and subsequently restoring activity which can be used to prevent premature clot
20 formation.

It is a further object of the invention to provide a kit suitable for use in the above-described method.

25 A more complete appreciation of the present invention and the advantages thereof will be readily understood by one skilled in the art from a reading of the description that follows.

In one embodiment, the present invention relates to a method of effecting the formation of
30 fibrin sealant at a body site. The method comprises: i) mixing, in a container means, an

aqueous solution comprising fibrinogen, Factor XIII and mature thrombin under conditions such that thrombin clotting activity is inhibited; and ii) applying a preparation resulting from step (i) to the body site under conditions such that thrombin clotting activity is restored and the fibrin sealant is formed.

In another embodiment, the present invention relates to a method of effecting the formation of fibrin sealant at a body site comprising: i) forming a suspension comprising a first phase which comprises fibrinogen and Factor XIII and a second phase which comprises thrombin, and ii) applying the suspension to the body site under conditions such that mixing of the fibrinogen, Factor XIII and thrombin is effected so that the fibrin sealant is formed.

In a further embodiment, the present invention relates to a kit for use in the preparation of a fibrin sealant. The kit includes an applicator comprising: i) a container means having disposed therein a solution comprising fibrinogen, Factor XIII and mature thrombin; and ii) an outlet means operably connected to said container means.

DETAILED DESCRIPTION OF INVENTION

The present invention relates to a method of delivering the components of a fibrin sealant (mature thrombin (as opposed to prothrombin) and the plasma-derived fibrinogen/Factor XIII precipitate) to a body site in a manner such that clot formation

is effected, and to a kit suitable for use in such a method. (The term "body site" as used herein includes the tissue in the area of a wound or incision as well as implantable tissues or components to be inserted into the area, e.g., vascular prostheses, bone or collagen pads.) In the description that follows, it will be appreciated that a combination of isolated forms of fibrinogen and Factor XIII can be used in place of the plasma-derived precipitate.

In the method of the present invention, a fibrinogen/Factor XIII-enriched precipitate and mature thrombin are mixed together under conditions such that thrombin and/or Factor XIII are/is inactivated (or under conditions such that thrombin is present in an active form but is rendered unavailable, as in the calcium depletion embodiment described below) and clotting thereby prevented. The mixture is then delivered to the body site under conditions such that the enzyme activity is restored (or thrombin availability restored).

In one embodiment, the mixture of thrombin and fibrinogen/Factor XIII precipitate is prepared in a low pH buffer (the clotting of fibrinogen by thrombin being inhibited by low pH (less than 5.5)). In this embodiment, thrombin activity is restored and clotting rapidly initiated upon neutralization of the mixture with a pharmaceutically acceptable buffer, or alternatively, upon contact of the mixture with the patient's own body fluids. In this embodiment, the fibrinogen/Factor XIII precipitate can be prepared at a low pH or, alternatively, a low pH buffer can be used to dissolve the plasma

precipitate and the lyophilized thrombin. In either case, the mixture can be transferred to a delivery container (such as a spray bottle or syringe) and applied to the body site directly, if conditions are such that the patient's body fluids are sufficient to increase the pH to a point where clotting occurs. Where conditions are such that the patient's body fluids are not sufficient to raise the pH of the precipitate/thrombin mixture to a point where thrombin activity is restored, a delivery device can be used that is designed such that, as the acidic mixture passes out of the device, it is contacted with buffer salts coated on an interior portion of the device. The buffer salts are selected such that when contact is made with the acidic mixture, dissolution occurs with the result that the pH is raised to a point where clotting takes place. For example, a syringe can be used as the delivery device (applicator), where the syringe is fitted with a disposable tip, the interior surface of which is coated with appropriate buffer salts. As the acidic mixture passes through the coated tip, the buffer (in the form, for example, of crystals or a gel) neutralizes the acidic mixture, thus restoring thrombin activity and effecting the formation of a clot at the desired site. Should clot formation occur in the tip, the tip can simply be removed and a new coated tip attached.

In another embodiment, the fibrinogen and Factor XIII precipitate/thrombin mixture can be prepared in a buffer that is depleted of calcium. Rapid clot formation requires the presence of calcium ions; thus, if the calcium is removed,

fibrin polymerization is inhibited (see Carr et al
Biochem J. (1986) 239:513; Kaminski et al J. Biol.
Chem (1983) 258:10530; Kanaide et al (1982) 13:229).
Calcium chelators (compounds such as sodium citrate
5 or ethylenediaminetetraacetic acid, which tightly
bind calcium and make it inaccessible) can be added
to the solution used to precipitate the fibrinogen
and Factor XIII and/or the dissolving buffer. To
restore activity, the container (for example, a
10 syringe) can be attached to a disposable sterile
tip, the interior surface of which is coated
internally with sufficient calcium salt to saturate
the chelator. As the free calcium concentration
increases upon passage of the mixture through the
15 tip, clotting is effected at the body site.

In a further embodiment, the clotting
activity of thrombin, in the precipitate/thrombin
mixture, can be inhibited using a photosensitive
inhibitor. For example, light sensitive cinnamoyl
20 derivatives can be used to inactivate thrombin, at
room temperature in the absence of light, for more
than 26 hours (Turner et al J. Am. Chem. Soc. 109:
1274-1275 (1987); Turner et al J. Am. Chem. Soc.
110: 244-250 (1988)). These same thrombin inhibitor
25 complexes can generate active thrombin within 1-2
seconds of irradiation (low intensity). These
inhibitors are known to form acyl-enzyme complexes
involving the active site serine hydroxyl (SER 195).
Upon irradiation, the cinnamoyl derivative undergoes
30 photoisomerization to release coumarin and
regenerate the active serine hydroxyl. Since
coumarin derivatives are not good thrombin
inhibitors, this phot cyclization reaction

effectively removes inhibitor from the enzyme solution. Thus, a solution of the fibrinogen/Factor XIII-enriched precipitate can be mixed with lyophilized inhibitor:thrombin complex in a dark environment (such as an opaque or colored syringe or container) and delivered to the wound site.

Activation of the enzyme and thus clot formation occurs upon delivery to the wound due to the exposure of the solution to normal room light.

Alternatively, activation can be controlled by a light source, for example, one built directly into the applicator, so that variations in lighting conditions will not result in variable clotting times.

Preferably, the clotting activity of thrombin, in the precipitate/thrombin mixture is inhibited by the use of a photosensitive inhibitor in combination with an irreversible inhibitor. Such "doubly-inhibited" thrombin offers several advantages including increased stability of the double inhibitor:thrombin complex and fibrinogen/Factor XIII precipitate which reduces premature clot formation during long term storage as well as during use. The doubly inhibited thrombin also provides for increased control of clot activation, increased clot strength and increased time for mixing with additives (such as, for example, bone granules, antibiotics or growth factors).

For example, thrombin activity can be inhibited by the use of the photosensitive inhibitor 4-amidino-phenyl-2-hydroxy-4-diethylamino-alpha-methylcinnamate hydrochloride, "I-1" and the

irreversible inhibitor D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone, "PPACK". I-1 inhibits approximately 99% of the thrombin activity, however, the residual 1% activity can not be reduced by additional I-1. This residual activity, which is sufficient to cause clotting after 15-20 minutes in the absence of light, can be reduced or titrated by the addition of the second inhibitor ("PPACK" in the present example). In the absence of activating irradiation (light of approximately 360 nm as provided by a Caulk UV Polymerization Unit), the doubly inhibited thrombin solution is subsequently added to the fibrinogen component and stored frozen, lyophilized or used within hours. Fibrin sealant prepared from this double inhibitor:thrombin complex and fibrinogen/Factor XIII precipitate can be maintained in the dark without clotting for over 2 hours. When the fibrin sealant is needed, the mixture can be dispensed onto the wound site or appropriate delivery container (such as a spray bottle or syringe) and activated by light to clot in less than 1-2 minutes.

Thrombin inhibited by the combination of such inhibitors may be useful for other applications. For example, the double inhibitor:thrombin complex can be used in situations requiring careful control of clotting activity or when thrombin is used alone to stop bleeding. In addition, the inhibited thrombin can be used to localize clotting activity (for example, eye surgery). The inhibited thrombin may also be used with other components or in other forms (for

example, with gel matrix or on solid support) to improve handling of delivery to the wound site.

5 In yet another embodiment, premature clot formation can be prevented prior to delivery of the fibrinogen and Factor XIII/thrombin mixture by physically separating the thrombin from the fibrinogen/Factor XIII precipitate. In this embodiment, physical separation is effected using a two-phase system. Liquids suitable for use in this
10 embodiment are non-miscible and readily separable into two phases. The two phases are mixed into a suspension before each application and delivered to the wound. Where conditions are such that the patient's body fluids extract the soluble component
15 of the nonaqueous phase, mixing occurs at the body site and clotting is thus initiated. If conditions will not elicit proper mixing of components, a delivery device can be used that is designed such that, as the suspension passes out of the device, it
20 is contacted with a solubilizing agent coated on an interior portion of the device. The solubilizing agent is selected such that when contact is made with the suspension, dissolution occurs with the result that mixing occurs to a degree where clotting
25 takes place. For example, a syringe is fitted with a disposable tip, the interior surface of which is coated with an appropriate phase transfer agent(s). As the suspension passes through the coated tip, the phase transfer agent (in the form, for example, of
30 crystals) assists in the mixing process, thus allowing clot formation. Should clot formation occur in the tip, the tip can simply be removed and a new coated tip attached.

The present invention also relates to a kit suitable for use in the above-described method of delivering fibrin sealant components to a wound site. In a preferred embodiment, the kit includes
 5 an applicator designed so as to permit mixing of the fibrinogen/Factor XIII precipitate and thrombin in a single system. The applicator can be one that permits the application at the body site of, for example, a film, or a thin line of the components of
 10 fibrin sealant. Alternatively, a pump or aerosol spray applicator can be used.

As suggested above, the applicator can, for example, take the form of a glass or plastic syringe with disposable tips. The shape of the tip
 15 will determine the form in which the components are delivered. A tip with a flat, broad end can be used to deliver a thin wide streak of fibrin sealant whereas a narrow tubular end can be used to deliver a round thread of sealant. Applying pressure to
 20 force the mixture through a tip constricted with, for example, a mesh screen can be used to produce a spray, resulting in a fine glaze of fibrin sealant. In another embodiment, particularly suitable for use with the above-described photosensitive thrombin
 25 inhibitor, the applicator can take the form of a pump or aerosol spray device having a built-in light source situated such that, as the sealant components exit the device, they are irradiated with the light. The wavelength of light used would depend on the
 30 photosensitizer.

The kit can be structured so as to include individual storage containers for the separate fibrin sealant components. The kit can also include

one or more other storage containers disposed within which are any necessary reagents, including solvents, buffers, etc.

5 The present invention will be understood in greater detail by reference to the following non-limiting Examples.

EXAMPLES

Example 1

10 A precipitate containing fibrinogen and Factor XIII was prepared as follows:

Four hundred fifty microliters of a stock 1 M zinc sulfate solution were added to 5 ml of anticoagulated (citrate phosphate dextrose adenine (CPDA-1)) human plasma. The solution was mixed well
15 without vortexing and centrifuged at 2,000 to 9,000 g for 5 minutes. The supernatant was decanted and discarded.

Inhibition of clotting and reactivation was achieved by any of the following methods:

20 A. Acid Inhibition - Lyophilized bovine thrombin was dissolved in citrate buffer (500 mM citric acid, 150 mM NaCl, and 20 mM EACA, pH 4.5) to a final concentration of 100 U/ml. Precipitated fibrinogen was dissolved in Tris buffer (50 mM Tris,
25 250 mM sodium citrate, 150 mM sodium chloride, 50 mM Arginine (Arg), and 20 mM ϵ -amino-caproic acid (EACA), pH 7.4) to a concentration of approximately 15.0 mg/ml. This fibrinogen stock was then diluted 25-fold in citrate buffer. The clotting time for
30 200 microliters of this fibrinogen solution plus 100 microliters thrombin exceeded 90 seconds in a Becton

Dickinson BBL Fibrosystem fibrometer under standard conditions indicating no clot formation. Addition of 70 microliters of 1N sodium hydroxide resulted in clot formation in 3.8 seconds (average of 10 samples).

The following procedures can be used for application to a wound site:

Where body fluids are sufficient to neutralize the acidic mixture of precipitate and thrombin, the mixture can be applied directly to the wound site. Alternatively, the delivery device can be connected to disposable tips coated internally with a neutralizing salt or gel (e.g. Tris). Neutralization of the acidic solution by the buffer salts activates thrombin and restores clotting activity.

B. Chelator Inhibition - Precipitated fibrinogen and lyophilized bovine thrombin were dissolved in Tris buffer (50 mM Tris, 250 mM sodium citrate, 150 mM sodium chloride, 50 mM Arg, and 20 mM EACA, pH 7.4) to a concentration of approximately 15.0 mg/ml and 100 U/ml, respectively. The fibrinogen stock solution was then diluted 25-fold in Tris buffer containing 500 mM sodium citrate. The clotting time for 200 microliters of this fibrinogen solution plus 100 microliters thrombin exceeded 90 seconds in a Becton Dickinson BBL fibrosystem fibrometer under standard conditions. Addition of 50 microliters of a 1M CaCl₂ solution resulted in clot formation in 1.8 seconds (average of 10 samples).

The following procedure can be used for application to a wound site:

5 The delivery device is connected to disposable tips coated internally with a calcium salt or gel. As the mixture passes through the tip, the molar excess of calcium saturates the chelator and clotting is thereby promoted.

10 C. Photosensitive Inhibition - In the absence of light, a 5 to 20-fold excess of 4-amidino-phenyl-2-hydroxy-4-diethylamino-alpha-methylcinnamate hydrochloride (Porter et al, J. Amer. Chem. Soc. 111:7616 (1989)) was added to thrombin in buffer (approximately 100 U/ml in 50 mM Tris, 250 mM sodium chloride, 250 mM sodium citrate, 15 20 mM EACA, 50 mM arginine (or urea), pH 7.4, final methanol concentration <10%). The inhibition was allowed to proceed for at least 1 hour at room temperature.

20 A minimal quantity of this solution was used to dissolve the precipitated fibrinogen/Factor XIII. This sealant required approximately 2 to 3 minutes illumination under standard operating lights to clot completely, whereas a sample mixture kept in the dark did not clot after 90 min.

25 The following procedures can be used for application to a wound site:

30 The photosensitive inhibitor-thrombin complex can be mixed with the precipitated fibrinogen/Factor XIII in a colored delivery device that does not transmit light of the activating wavelengths. Delivery of the mixture to an illuminated wound site results in clot formation.

D. Two Phase Suspension - Lyophilized bovine thrombin is dissolved in an emulsifying agent to a final concentration of about 100 U/ml. Precipitated fibrinogen/Factor XIII is dissolved in
 5 a minimal volume of buffer (50 mM Tris, 150 mM sodium chloride, 250 mM sodium citrate, 20 mM EACA, 50mM Arg, pH 7.4). A suspension of the immiscible liquids is formed. On a wound surface, body fluids may be sufficient to dissolve both components and
 10 promote proper mixing and clot formation.

Example 2

A fibrin sealant was prepared using the two enzyme inhibitors as follows. All inactivation and transfer steps were performed in the absence of
 15 activating light (366 nm), using a dark room illuminated by a red light.

Thrombin was inactivated sequentially using 1) the light sensitive inhibitor 4-amidinophenyl-2-hydroxy-4-diethylamino-alpha-methylcinnamate hydrochloride and 2) D-Phenylalanyl-L-prolyl-L-arginine Chloromethyl Ketone, PPACK.
 20 Bovine thrombin (0.1 ml of 1,000 units/ml Armour Thrombinar) was mixed with 0.8 ml of 50 mM tris buffer, pH 7.3, and 0.1 ml of 31.25 µg/ml inhibitor
 25 in 2.5% ethanol, 50 mM tris, pH 7.3. The solution was incubated at room temperature for 2 hours.

Residual thrombin activity of the thrombin:inhibitor-1 complex (approximately 1% original activity) was inactivated by titration with
 30 PPACK until residual activity was such that the resulting fibrin sealant could be maintained in the

dark for 2-4 hours without premature clotting. The
above solution (0.1 ml) was mixed with 0.01 ml of
2.5 µg/ml PPACK at room temperature and thrombin
activity of the thrombin:inhibitor-2 was negligible
5 within minutes.

Fibrin sealant was prepared by mixing 1 ml
fibrinogen stock (e.g. single unit human
cryoprecipitate) with 0.1 ml of the
thrombin:inhibitor-2 in the absence of activating
10 light (e.g. opaque container) and transferred to an
amber syringe or spray container for delivery.

Clotting activity was easily controlled
using light. In the absence of activating light,
the mixture was stable for at least 2 hours at room
15 temperature before clotting occurred. A 20 second
pulse of light (that is, light of 360 nm, by Caulk
MAX polymerization unit) at a distance of less than
5 cm from the dispensed solution resulted in
clotting within seconds.

20

* * * *

The entire contents of each of the
references cited above are hereby incorporated by
reference.

While the present invention has been
25 described in some detail for purposes of clarity and
understanding, it will be clear to one skilled in
the art from a reading of this disclosure that
various changes can be made in form and detail
without departing from the true scope of the
30 invention.

WHAT IS CLAIMED IS:

1. A method of effecting the formation of fibrin sealant at a body site comprising:
 - i) mixing, in a container means, an aqueous solution comprising fibrinogen, Factor XIII and mature thrombin under conditions such that thrombin clotting activity is inhibited; and
 - ii) applying a preparation resulting from step (i) to said body site under conditions such that thrombin clotting activity is restored and said fibrin sealant is formed.
2. The method according to claim 1 wherein step (i) is carried out at a pH of less than 5.5, whereby thrombin clotting activity is inhibited.
3. The method according to claim 2 wherein, in step (ii), the pH of said preparation resulting from step (i) is increased such that thrombin clotting activity is restored.
4. The method according to claim 3 wherein the pH of said preparation resulting from step (i) is increased upon contact of said preparation with body fluids of said patient present at said body site.
5. The method according to claim 3 wherein, in step (ii), the pH of said preparation resulting from step (i) is increased upon contact with a buffer capable of increasing the pH.

6. The method according to claim 1 wherein said solution of step (i) includes an amount of a photosensitive inhibitor of thrombin clotting activity sufficient to inhibit thrombin clotting activity.

7. The method according to claim 6 wherein, in step (ii), said preparation resulting from step (i) is irradiated with light of a wavelength that inactivates said photosensitive inhibitor, whereby thrombin clotting activity is restored.

8. The method according to claim 1 wherein said solution of step (i) includes an amount of a photosensitive inhibitor of thrombin clotting activity and an amount of an irreversible inhibitor of thrombin clotting activity sufficient to inhibit thrombin clotting activity.

9. The method according to claim 8 wherein, in step (ii), said preparation resulting from step (i) is irradiated with light of a wavelength that inactivates said photosensitive inhibitor, whereby thrombin clotting activity is restored.

10. The method according to claim 1 wherein the conditions of step (i) are such that thrombin clotting activity is inhibited by the absence of calcium ions sufficient to effect fibrin sealant formation.

11. The method according to claim 10 wherein, in step (ii), the conditions are such that calcium ions sufficient to effect fibrin sealant formation are present.

12. The method according to claim 10 wherein said solution of step (i) includes an amount of a chelator of calcium sufficient to reduce free calcium ions in said solution to a level insufficient to effect fibrin sealant formation.

13. The method according to claim 11 wherein, in step (ii), said preparation resulting from step (i) is contacted with an amount of calcium ions sufficient to effect fibrin sealant formation.

14. A method of effecting the formation of fibrin sealant at a body site of a patient comprising:

i) forming a suspension comprising a first phase which comprises fibrinogen and Factor XIII and a second phase which comprises thrombin; and

ii) applying said suspension to said body site under conditions such that mixing of said fibrinogen, Factor XIII and thrombin is effected so that said fibrin sealant is formed.

15. The method according to claim 14 wherein, in step ii, said mixing occurs in a body fluid of said patient present at said body site.

16. The method according to claim 14 wherein, in step (ii), said suspension of step (i) is contacted with a phase transfer agent that effects mixing of said first and said second phase such that said fibrin sealant is formed.

17. A kit for use in the preparation of a fibrin sealant comprising an applicator that comprises:

i) a container means having disposed therein a solution comprising fibrinogen, Factor XIII and mature thrombin; and

ii) an outlet means operably connected to said container means.

18. The kit according to claim 17 wherein said solution further comprises a calcium chelator.

19. The kit according to claim 18 wherein said outlet means has a calcium salt disposed therein.

20. The kit according to claim 17 wherein said solution has a pH of less than about 5.5.

21. The kit according to claim 20 wherein said outlet means has a neutralizing buffer salt disposed therein.

22. The kit according to claim 17 wherein said solution further comprises a photosensitive inhibitor of thrombin clotting activity and wherein said container means and said outlet means are

constructed of a material that does not transmit light of a wavelength to which said inhibitor is sensitive.

23. The kit according to claim 22 wherein said outlet means includes a source of light that, when activated, emits light at a wavelength that inactivates said photosensitive inhibitor.

24. The kit according to claim 17 wherein said solution further comprises a photosensitive inhibitor of thrombin clotting activity and an irreversible inhibitor of thrombin clotting activity and wherein said container means and said outlet means are constructed of a material that does not transmit light of a wavelength to which said inhibitor is sensitive.

AMENDED CLAIMS

[received by the International Bureau on 6 September 1993 (06.09.93);
original claims 8 and 24 amended;
remaining claims unchanged (2 pages)]

6. The method according to claim 1 wherein said solution of step (i) includes an amount of a photosensitive inhibitor of thrombin clotting activity sufficient to inhibit thrombin clotting activity.

7. The method according to claim 6 wherein, in step (ii), said preparation resulting from step (i) is irradiated with light of a wavelength that inactivates said photosensitive inhibitor, whereby thrombin clotting activity is restored.

8. The method according to claim 1 wherein said solution of step (i) includes an amount of a photosensitive inhibitor of thrombin clotting activity and an amount of a second inhibitor of thrombin clotting activity sufficient to inhibit thrombin clotting activity.

9. The method according to claim 8 wherein, in step (ii), said preparation resulting from step (i) is irradiated with light of a wavelength that inactivates said photosensitive inhibitor, whereby thrombin clotting activity is restored.

10. The method according to claim 1 wherein the conditions of step (i) are such that thrombin clotting activity is inhibited by the absence of calcium ions sufficient to effect fibrin sealant formation.

constructed of a material that does not transmit light of a wavelength to which said inhibitor is sensitive.

23. The kit according to claim 22 wherein said outlet means includes a source of light that, when activated, emits light at a wavelength that inactivates said photosensitive inhibitor.

24. The kit according to claim 17 wherein said solution further comprises a photosensitive inhibitor of thrombin clotting activity and a second inhibitor of thrombin clotting activity and wherein said container means and said outlet means are constructed of a material that does not transmit light of a wavelength to which said inhibitor is sensitive.

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61M 31/00

US CL :604/49; 606/214

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 604/49; 606/214; 604/46, 49, 56, 82-92, 416, 290; 128/DIG22; 606/2, 3, 8, 11; 602/48-50

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 4,735,616 (EIBL ET AL.) 05 April 1988. See the entire document.	17
X	US, A, 4,427,651 (STROETMANN) 24 January 1984. See the entire document.	17
X	US, A, 4,359,049 (REDL ET AL.) 16 November 1982. See the entire document.	1, 17
X	US, A, 4,453,939 (ZIMMERMAN ET AL.) 12 June 1984. See the entire document.	14-16
X	US, A, 4,359,463 (ROCK) 16 November 1982. See the entire document.	17, 18

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 11 MAY 1993	Date of mailing of the international search report 29 JUN 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer MARK BOCKELMAN
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of the American Chemical Society, 1987, A.D. Turner et al "photochemical Activation of Acylated Thrombin" pages 1274-1275. See the entire document.	17, 22, 23
Y	US, A, 2,533,004 (FERRY) 05 December 1950. See the entire document.	17, 20
Y	Arch. Biochem. Biophy (1951)y Koloman Laki, "The Polymerization of Proteins. The actionof Thrombin and Fibrinogen pages 317-324. See the entire document.	17, 20